

Applicant: Werner *et al.*
International Appl. No.: PCT/EP2003/013021
International Filing Date: November 20, 2003
U.S. Appl. No. 10/535,763; Filed May 20, 2005
Amdt. dated 05/01/2006

Amendments to the Specification:

Please enter into the specification the paper and computer-readable forms of the Sequence Listing submitted concurrently herewith.

Please replace the first paragraph on page 20 of the specification with the following paragraph:

Another protein switch contemplated under the invention may rely on posttranslational modification of one or more additional heterologous nucleic acid(s). There are many possible implementations of such protein switches that could operate by controlling steps such as polypeptide folding, oligomer formation, removal of targeting signals, conversion of a pro-enzyme into an enzyme, blocking enzymatic activity, etc. For example, delivery of a site-specific protease into cells of a multi-cellular organism may trigger a cellular process of interest if a genetically-engineered host specifically cleaves a pro-enzyme, thus converting it into an active enzyme, if a product is targeted to a particular cellular compartment because of the host's ability to cleave or modify a targeting motif, or if a product is specifically mobilized due to the removal of a specific binding sequence. Cleavage of a translational fusion protein can be achieved via a peptide sequence recognized by a viral site-specific protease or via a catalytic peptide (Dolja *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89, 10208-10212; Gopinath *et al.*, 2000, *Virology*, 267, 159-173; US5162601; US5766885; US5491076). Other examples of site-specific proteases applicable to this invention are mammalian enterokinases, for example, human enterokinase light chain which recognizes the sequence DDDK-I (SEQ ID NO:1) (Kitamoto *et al.*, 1994, *Prtoc. Natl. Acad. Sci.*, 91, 7588-7592), and specifically cleaves Lys-Ile bonds; viral proteases, like Hc-Pro (Carrington JC & Herndon KL, 1992, *Virology*, 187, 308-315) which catalyses proteolysis between the Gly-Gly dipeptide but requires 4 amino acids for the recognition of the cleavage site; site-specific protease of Semliki Forest Virus (Vasiljeva *et al.*, 2001, *J Biol Chem.*, 276,

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30786-30793); and proteases involved in polyubiquitin processing, ubiquitin-carboxy-terminal hydrolases (Osava *et al.*, 2001, *Biochem Biophys Res Commun.*, 283, 627-633).

Please replace the paragraph that bridges pages 21-22 of the specification with the following paragraph:

The polypeptide of interest can be applied externally to target cells of said plant using a covalent fusion or non-covalent interaction with a membrane translocating sequence. Many examples of membrane translocating sequences (MTS), natural and synthetic, are known in the art. They are widely used as fusions with peptide drugs and therapeutic proteins in order to increase their cell membrane permeability. An MTS may be a simple amino acid repeat, for example a cationic peptide containing eleven arginines RRRRRRRRRRRR (SEQ ID NO:2) (Matsushita *et al.*, 2001, *J. Neurosci.*, 21, 6000-6007). Another cationic MTS is a 27 amino acid long transportan (GWTLSAGYL LGKINLKALA ALAKKIL; SEQ ID NO:3) (Pooga *et al.*, 1998, *FASEB J.*, 12, 67-77). It is very likely that such peptides, for their penetration of the cell, exploit the asymmetry of the cellular plasma membrane where the lipid monolayer facing the cytoplasm contains anionic phospholipids (Buckland & Wilton, 2000, *Biochim. Biophys. Acta/Mol. Cell. Biol. Of Lipids*, 1483, 199-216). Certain proteins also contain subunits that enable their active translocation across the plasma membrane into cells. To such domains belongs the basic domain of HIV-1 Tat₄₉₋₅₇ ((RKKRRQRRR; SEQ ID NO:4) (Wender *et al.*, 2000, *Proc. Natl. Acad. Sci. USA*, 97, 13003-13008), Antennapedia₄₃₋₅₈ (RQIKIWFQNR RMKWKK; SEQ ID NO:5) (Derossi *et al.*, 1994, *J. Biol. Chem.*, 269, 10444-10450), the Kaposi Fibroblast Growth Factor MTS (AAVALLPAVL LALLAP; SEQ ID NO:6) (Lin *et al.*, 1995, *J. Biol. Chem.*, 270, 14255-14258); the VP22 MTS (Bennet, Dulby & Guy, 2002, *Nat. Biotechnol.*, 20, 20; Lai *et al.*, 2000, *Proc. Natl. Acad. Sci. U S A*, 97, 11297-302); homeodomains from the *Drosophila melanogaster* Fushi-tarazu and Engrailed proteins (Han *et al.*, 2000, *Mol Cells* 10, 728-732). It was shown that all these positively charged MTSs are able to achieve cell entry by

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themselves and as fusions with other proteins like GFP (Zhao *et al.*, 2001, *J. Immunol. Methods*, 254, 137-145; Han *et al.*, 2000, *Mol Cells*, 10, 728-732), Cre recombinase (Peitz *et al.*, 2002, *Proc. Natl. Acad. Sci. USA*, 4489-4494) in an energy-independent manner. However, the fusion is not necessarily required for protein transport into the cell. A 21-residue peptide carrier Pep-1 was designed (KETWWETWWTEWSQPKKKRKV; SEQ ID NO:7) which is able to form complexes by means of non-covalent hydrophobic interactions with different types of proteins, like GFP, b-Gal, or full-length specific antibodies. These complexes are able to efficiently penetrate cell membranes (Morris *et al.*, 2001, *Nature Biotechnol.*, 19, 1173-1176). The list of MTS can be continued and, in general, any synthetic or naturally occurring arginine-rich peptide can serve for practicing this invention (Futaki *et al.*, 2001, *J. Biol. Chem.*, 276, 5836-5840).